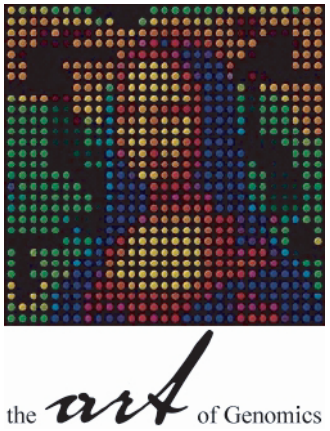
OpArray® User Manual

Version 2.0



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Operon Technologies, Inc.

1000 Atlantic Ave., Alameda, CA 94501, USA

Ph: 800-688-2248 510-865-8644

Email: dna@operon.com Web: www.operon.com

Operon GmbH

Nattermannallee 1, D-50829 Köln, Germany

Customer Service: 00800-OP ORDERS (00800-67 67 33 77)

Tech Support: 00800-OP SUPPORT (00800-677 877 678)

Email: oligo@operon.com Web: www.operon.com

Notes

References

These protocols are based on the following sources:

1) The MGuide – http://cmgm.stanford.edu/pbrown/mguide

2) Eisen MB; Brown PO. DNA Arrays for Analysis of Gene Expression. *Methods in Enzymology*, 1999, 303:179-205.

3) DeRisi Lab Protocols – http://www.microarrays.org

VIII. Troubleshooting

Problem	Probable Causes	Solution
Lov yield cDNA probe	Source RNA degraded	Run RNA on an agarose gel to insure RNA integrity
	Reagents not RNase free	Run cDNA out on a gel +/-
	RNA impure	Check RNA purification procedure for errors. Repurify RNA
	Nucleotides degraded	Make frozen aliquots of nucleotides
	Missing reagent	Check reaction and repeat
	Inactive reverse transcriptase	Retry reaction with fresh enzyme
	Failure to neutralize cDNA prior to QIAquick purification	Add sodium acetate to sample following treatment with hydroxyalamine
Inefficient dye incorporation	Cy Dye pack inactive	Store dried Cy aliquots from DMSO in the absence of light at 4°C in a desiccator
	pH of Carbonate buffer not equal to 8.5-9.0	Test pH of 100mM carbonate buffer
High slide background	Poor probe quality	Take a spectrophotometer reading of the probe prior to hybridization. See low yield cDNA probe/inefficient dye incorporation
	Insufficient blocking of the slide	Insufficient washing following hybridization can result in spots containing "comet tails," or smeary spots. This can be avoided by thoroughly dunking slides during these washes
Very low spot intensities	Poor probe quality	Take a spectrophotometer reading of the probe prior to hybridization. See low yield cDNA probe/inefficient dye incorporation
	Scanning intensity too low	Increase scanning power

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I. Product Description

In the past few years, as genome project sequencing information has become widely available, the popularity and usefulness of DNA microarrays has grown exponentially. One widely used application for these "gene chips" has been the monitoring of gene expression. In response to the demand for a reliable tool for this purpose, Operon has developed the OpArray, a microarray containing optimized long oligonucleotide sequences.

Genes with a high degree of homology can present cross hybridization problems that lead to false negatives and positives. Currently available genomic primer sets provide oligos that are used in the amplification of DNA products, spanning the full open reading frame (ORF) from start to stop codon. In contrast, Operon designs and synthesizes oligos representing a 70nt region of the gene of interest. These sequence optimized 70mers, or OPTs, are bioinformatically designed to minimize cross hybridization. In addition, sequences are T_m normalized and selected to minimize secondary structure.

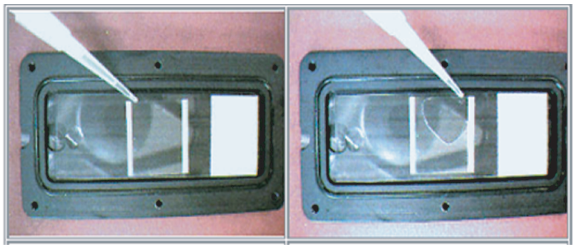

Figure 2: Applying the Probe

- Lifter slips make pipetting the probe very easy. It wicks under the cover slip with no bubbles (slide B)
  Note: New cover slips are longer and should be placed on the slide 90° to the one shown in the picture above.
- Place the slide into the hybridization chamber
- Pipette 15µl 3 x SSC near one of the edges of the slide, away from the cover slip
- Tighten the screws for the chamber cover and place the assembly in a 67°C water bath for 6-12 hrs.

VII. Post Treatment

Wash 1: 1 x SSC, 0.03% SDS
Wash 2: 0.2 x SSC
Wash 3: 0.05 x SSC

- Carefully remove the chamber and dry it with paper towels
  Note: If you have several chambers to process, dry them off and remove the screws, but refrain from cracking them open until all the chambers are ready to open.
- Remove the slide from the chamber and place it into a slide rack to be washed
- Gently plunge the rack up and down several times to get the cover slip to fall off the slide. Wash for 2-3 min.
  Note: Lifter cover slips are reusable and should be washed in 70% EtOH prior to use.
- Always spin-dry the slides for 5 min.
  Note: If using 50ml conical tubes to spin slides with the table top centrifuge, be sure that the slide is orientated with the label side down.

VI. Hybridization Protocol

A. Probe Preparation

Note: The probe can be dried down to an appropriate volume. Add SDS last, just prior to denaturation, to avoid precipitation. Use 12-15µl probe mix per hybridization. If using Lifter slips, prepare 20µl of probe per hybridization.

Stock	**Final [ ]**	**µl**
20X SSC	2.6X	1.9
*Poly dA/Cot1 (10µg/µl)	10µg	1
cDNA probe mix	~1µg	10.6
2%SDS	0.2%	1.5

*Include Cot 1 DNA for Human Probe only

- Denature at 95°C for 2 min.
- Spin probe briefly

  Note: This serves two purposes:
  1) to pellet condensation
  2) to cool probe

B. Applying Probe

- Align hybridization slide with reference slide

  Note: Spots can be visualized by breathing on the slide to fog the surface.
- Place clean lifter cover slip, Teflon side down, over the array area

  Note: Lifter cover slips have Teflon edges along the two opposing outer edges. This provides an advantage over traditional cover slips in that it allows for better diffusion of probe. In addition, air bubbles under the slip are more easily avoided.
- Place pipette tip along an open edge of the cover slip and slowly pipette the probe out of the tip. Capillary action will draw the probe mix under the slip
- Please note the hydration spot near the left side of slide A (added last, just prior to loading the chamber). See figure 2

II. Kit Contents

All OpArrays are shipped with the following materials:

- OpArray reference slide
- Floppy disk containing the following files:
  - array layout
  - gene list
  - OpArray QC hybridization images
  - Readme.txt - array parameters

III. Reagents and Equipment Needed

Description	Supplier	Catalog #	Price
OmniScript® (200 units)	QIAGEN	205111	$155.00
5-(3-aminoallyl)-2'-deoxyuridine 5' triphosphate	Sigma	A0410	5mg for $277.40
Cy3 Mono-Reactive dye pack	Amersham	PA23001	$170.00
Cy5 Mono-Reactive dye pack	Amersham	PA25001	$170.00
Microcon YM-30 (24)	Millipore	42409	$64.00
QIAquick PCR Purification Kit (50)	QIAGEN	28104	$68.00
Poly dA (18mer)	Operon Technologies	Custom Synthesis	Get Quote
Human Cot1 DNA	Several	N/A	N/A
Hybridization Chamber	Operon Technologies	Get Quote	Get Quote
Hydroxylamine	Sigma	H2391	$22.60/100g
20X SSC	Sigma	S-6639	$36.30/liter
SDS	Several	N/A	N/A
Slide Rack	Several	N/A	N/A
Slide Chamber	Several	N/A	N/A
Slide Scanner	Several	N/A	N/A
Analysis Software	Several	N/A	N/A

prices subject to change May 2001

QC Probe

The quality of the probe may be checked by scanning it in a spectrophotometer (200-700nm).

Peak	Sample
260nm	cDNA
550nm	Cy3
649nm	Cy5

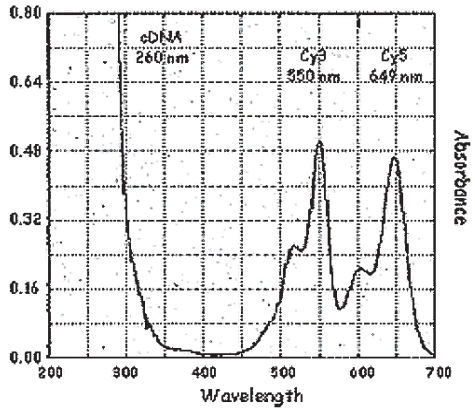

Figure 1: Sample Spectrophotometer Reading

Note:

i) If performing the QC scan, it is essential that all unconjugated dye is removed from the sample. The QIAGEN PCR Purification Kit is sufficient for this. If another kit is used for the last cleanup step, insure that dye removal is sufficient so the QC scan doesn't detect dye carry over.

ii) For dye conjugation, DMSO does not interfere with the conjugation reaction. Hence it is not necessary to dry down the dye aliquots if they are to be used directly. Simply re-suspend the cDNA in Carbonate buffer and add the dye in DMSO directly.

Protocol for QIAGEN PCR Purification Kit:

- Add 35µl 100 mM NaOAc, pH 5.2 to each reaction
- Combine reactions in 1 tube
- Add 500µl PB Buffer
- Apply sample to QIAquick column
- Spin for 30-60 sec., ~13,000 RPM (>10,000 x g)
- Discard flow-through, add 750µl PE buffer
- Spin for 30-60 sec.
- Discard flow-through
- Spin for 1 min.
- Place column in new tube
- Add 50µl EB Buffer or H_2O to membrane
  Note: Eluted volume is typically around 48µl. You may choose to elute with 1/2 x EB buffer and reduce the volume for hybridization in the spin vac.
- Spin for 1 min.

IV. Handling and Storage Conditions

Slides should be handled with gloves to avoid smudges and dirt. Store slides desiccated at 4°C in the absence of light. Store oligo aliquots at -20°C.

V. Reverse Transcription/ Labeling Protocol

This protocol utilizes Mono-Reactive Cyanine dyes to label cDNA *after* reverse transcription. Incorporation of a nucleotide containing an alkyl amino group allows post RT conjugation. Although there are other labeling methods available, we have found this protocol to be simple and effective.

Aliquotting:

50X dNTP Mix

dNTP	1x	50x	Stock []	Volume
dATP	500 µM	25mM	100mM	10µl
dCTP	500 µM	25mM	100mM	10µl
dGTP	500 µM	25mM	100mM	10µl
dTTP	300µM	15mM	100mM	6µl
aa-dUTP	200µM	10mM	100mM	4µl

Dye Packs

- Resuspend Cy dyes in 72 µl DMSO
- Aliquot 4.5 µl to 16 tubes
- Lyophilize in Speed Vac
- Store sample in the absence of light at 4°C in a desiccator

A. Reverse Transcription Reaction

Note: 2µg of mRNA should make enough probe for 2-3 hybridizations.

RNA mix	[ ]	µl
Oligo dT	5mg/ml	1
mRNA	1 to 2µg	14.5

- Incubate at 70°C for 10 min.
- Chill on ice
- Add the following reagents to the tube

Stock	[ ]	µl
10X Buffer	10X	3
aa-dUTP/dNTPs	50x	0.6
OmniScript		1.9
Water		9

- Incubate at 37°C for 2 hrs.

B. Hydrolysis of RNA

- Mix 10µl 1N NaOH and 10µl 0.5M EDTA
- Add to RT reaction
- Incubate for 15 min. at 65°C
- Neutralize 25µl 1M Tris ph 7.4, or 25µl 1M HEPES pH 7.0

C. Reaction Cleanup

- Place 450µl H_2O in Microcon 30
- Add reaction mix
- Spin at 11,000 x g for 12 min.
- Remove flow-through
- Repeat wash 2 x with H_2O
- Invert microcon in a new tube and elute by spinning 11,000 x g for 20 sec. The volume may range from 4µl to 40µl
- Dry down in spin vac and store at –20°C

D. Coupling of Cy Dye

- Resuspend cDNA in 4.5µl of H_2O
- Resuspend aliquot of Cy dye in 4.5µl 0.1 M Carbonate buffer, pH 8.5-9.0 (use Bicarbonate to adjust the pH)
- Mix cDNA and Cy dye
- Incubate in the dark for 1 hr. at room temperature

E. Quench Reaction

- Quench any un-reacted Cy dye by adding primary amines
- Add 4.5µl 4M Hydroxylamine
- Incubate for 15 min. in the dark at room temperature

F. Reaction Cleanup II and Hyb Prep

- Cy3 and Cy5 reactions can be combined and cleaned up together or separately. Removal of free dye can be performed with either gel filtration via spin column or Pasteur pipette, or with various kits, such as QIAGEN PCR Purification Kit. The QIAGEN PCR Purification Kit works well for this step. However, it is important to keep in mind that the DNA binding curve for silica, on which this kit is based, is favorable at low pH but falls off precipitously around pH 8. Thus it is essential that the pH of the reaction is below 7.5 before it attaches to the QIAGEN membrane.